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"Modulation of rye rDNA chromatin conformation induced by high temperature stress"

Report – Bolsa de integração na Investigação

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Modulation of rye rDNA chromatin conformation induced by high temperature stress

Abstract

Epigenetic modulation has been correlated with expression patterns alterations involved in plant response to stress. However, the influence of abiotic stress, namely high temperature, in chromatin organization of plant nuclei is poorly known. The aim of this work was the analysis of rDNA chromatin organization in interphase nuclei of rye root-tip and coleoptiles from high temperature treated (HTS) and untreated plantlets, through Fluorescent In Situ Hybridization (FISH) using rDNA probes. Rye seeds were germinated in controlled conditions and HTS treated plantlets were subject to a period of 4 hours at 40^{o}C. FISH was performed successfully using a new rDNA probe produced by PCR that can replace with advantage the pTa71 probe and the results obtained with both probes revealed significant differences in rDNA chromatin organization patterns between control and HTS in interphase nuclei from both tissues analyzed. The detected rDNA chromatin decondensation provoked by HTS may be involved in a greater potential for transcription necessary to the amendment induced by HTS and emphasize the role of chromatin epigenetic remodelling in plant adjustment pathways under stress conditions.

Modulação da conformação da cromatina ribossomal de centeio induzida pelo stress de alta temperatura

Sumário

A modulação epigenetica tem sido correlacionada com alterações de padrões de expressão em plantas face à resposta contra stress ambientais. No entanto, a influência de stresses abióticos, particularmente de temperaturas elevadas, sobre a organização da cromatina ainda é pouco conhecida. Deste modo, o objectivo deste trabalho consiste na análise da organização da cromatina ribossomal em núcleos interfásicos de ápices e coleóptilos de centeio. Foram observadas plantas com tratamento (HTS) e sem tratamento com recurso à hibridação In Situ (FISH), usando sondas especificas para rDNA. As sementes de centeio foram germinadas em condições controle e sujeitas a um período de 4 horas a 40^{o}C (tratamento HTS). A técnica FISH foi executada com sucesso usando uma nova sonda, produzida por PCR, que substitui com vantagem a sonda pTa71, sendo os resultados obtidos com ambas iguais, revelando diferenças significativas no padrão de organização da cromatina ribossomal entre núcleos interfásicos controle e HTS dos distintos tecidos analisados. A descondensação da cromatina ribossomal induzida pelo tratamento HTS pode estar envolvida no potencial de transcrição necessário ao ajuste provocado pela exposição a altas temperaturas, sublinhando o papel dos factores epigeneticos sobre a remodelação na cromatina em plantas expostas a condições de stress.

Introduction

Following human evolution, plants have always taken a prominent position. Since its medical properties to the most basic propose, feeding, their knowledge have taken a huge importance throughout civilizations growth and in our days scientific research. Due to its strong socio-economic importance, cereals are particularly well known species in field and laboratory, being an example rye. Rye belongs to *Triticeae* and is close related to barley and wheat. Is cultivated in large scale to produce grain and forage and is tolerant to acid soils and very resistant to cold and dry climates (Baier, 1994).

Several environmental parameters are crucial for plant development, as light, oxygen, carbon dioxide, nitrogen, nutrients, water and temperature. Changes of those parameters lead to plant stress responses. Gene expression modulation induced by stress is decisive for plant adaptation and is influenced by the structure and organization of chromatin, which corresponds to the association between DNA and proteins. Chromatin comprehends a heterochromatic (het) fraction that retains a condensed conformation during all interphase, and a euchromatic one, characterized by a lower compactation level, corresponding mainly to coding sequence-rich regions of the genome (Redi *et al.*, 2001; Avramova, 2002). The het fraction is largely composed of repetitive sequences that can be organized into small islands and is divided in constituent het that is always condensed in all cell types of an organism, and facultative het that is condensed only in certain cell types (Kunze *et al.*, 1996; Weichenhan *et al.*, 1998; Avramova, 2002).

Chromatin conformation and gene transcriptional status are regulated by epigenetic processes, associated to changes in histones variants and post-translational modifications and DNA methylation (Chinnusamy & Zhu, 2009). DNA sequences associated with heterochromatin are normally hipermethylated at cytosine residues and transcriptionaly inactivate. In Arabidopsis, the fine mapping of methylated cytosines showed that the level of DNA methylation increases from euchromatin to pericentromeric heterochromatic fraction of the genome. Histone post-translational modifications as acetylation, phosphorylation and ubiquitination are usually associated with transcriptional activity, while biotinylation and sumoylation are associated with expression repression (Chinnusamy & Zhu, 2009).

The ribosomal RNAs are key players in the production of ribosomes. According to the Svedberg coefficient, in plants rRNAs are referred to as 5S, 5.8S, 18S and 25S and result from the removal of introns (splicing) of a single transcribed sequence, 45S, encoded by the rDNA units (Sumner, 1990; Pederson and Politz, 2000; Helsop-Harrison, 2000). Those units include also a non-transcribed intergenic spacer and are clustered in long tandem arrays, typically spanning several megabases, in the

Nucleolar Organizing Regions (NORs) (Fig. 1). 45S rRNA transcription produces the nucleoli in interphase, 5S rRNA fragments are transcribed from other sites of the genome, and ribosomes are assembled within the nucleolus. As a practical matter, in the literature rDNA refers only to 45S rDNA (McClintock, 1934; review in Pikaard, 2002).

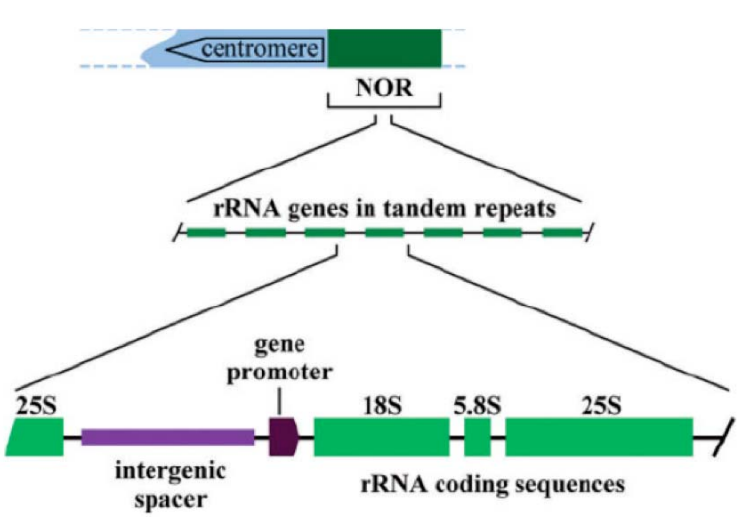

Fig.1 - Organization of the rDNA units at NORs (adapted from Neves *et al., 2005.)*

In late prophase, nucleoli disappear due to the interruption of gene transcription during mitosis. However, the result of NOR loci activity in interphase can be also visualized in the following metaphase as a second constriction (McClintock, 1934) reflecting the rDNA decondensation state during the previous interphase that allowed transcription machinery access (Jimenez *et al*., 1988).

In active plant cells, although the number of copies of rRNA genes is much higher than in animal cells, the number of active genes in the nucleolus is similar in both types of cells. In most plants only a short fraction of rRNA genes are actively transcribed, as demonstrated in Pisum sativum, where only 5% of the units are transcribed (González-Melendi *et al*., 2001). Accordingly, a small fraction of rRNA genes of one locus appears to be sufficient to supply all ribosomal machinery, suggesting that most rDNA units remain transcriptionally silent (Caperta *et al*. 2002). Moreover, homologous NORs usually have a similar expression behavior, namely both are transcribed or silenced (Zurita *et al*., 1999).

In many species, rDNA inactivation seems to involve a high level of rDNA chromatin condensation. In cereal nuclei, the condensed rDNA fraction is observed as dense chromatin knobs at the periphery of the nuclear area (perinucleolar localization) through in situ hybridization using a rDNA probe (Leitch *et al*., 1992; Delgado *et al*., 1995; Shaw & Jordan, 1995; Morais-Cecílio *et al*., 2000; Pontes *et al*., 2003). As showed in P. sativum, the perinucleolar blocks can decondense, being this change directly linked to a nucleolar volume increase (Shaw *et al*., 1993). Several recent findings reinforce the idea that interphasic perinucleolar condensation of rDNA chromatin is clearly linked to the silencing of rRNA genes, as suggested by BrUTP incorporotion detection (Silva *et al*., 2008). Those results are in agreement with the current model of chromatin organization NOR, in which some of the rRNA genes are condensed and inaccessible while another part is transcribed (Leitch *et al*., 1992; Morais-Cecílio *et al*., 2000; Silva *et al*., 2008).

Rye genome is characterized by seven pairs of chromosomes with the 45S rRNA genes clustered in one pair (1R). Each rye NORs are characterized by a perinucleolar condensed block which corresponds to the proximal region of the NOR. By sequential silver staining and in situ hybridization it was showed that untranscribed rDNA block resides at the centromere proximal NOR domain, so the descondensed region present into the nucleolus is telomere proximal (Neves *et al*., 2005).

This work's aim is to identify and characterize changes in ribosomal chromatin conformation observed in rye interphase nuclei from root-tips and coleoptiles of plants exposed to high temperature stress, through Fluorescente In Situ Hybridization (FISH) using rDNA probes.

Material and Methods

Rye cv. Imperial root-tip and coleoptile squashes used were obtained as described:

- Seeds were placed into Petri boxes with filter paper saturated with distillated water for 3 days at 4ºC in the dark, to synchronize seed germination.
- The seeds were then transferred to a growth chamber at 20ºc-day-14h / 10ºC-nigth-10h for 3 days;
- Plants subject to high temperature stress (HTS) were exposed to a temperature increase during 7 hours, starting at 20ºC until 40ºC, being this temperature kept for 4 hours;
- After this period, control and HTS root-tips and coleoptiles were fixed in ethanol: acetic acid (3:1 vol/vol) for 16 hours and then fresh fixative was changed and the material was kept at -20ºC. In coleoptile material, fresh fixative was changed 3 times more before storing until further use. Control root-tips were also subjected to a ice treatment to induce c-metaphases by immersion in ice-cold water for 24 hours prior to fixation;
- Root-tip and coleoptile tissues were washed for 2x 10 min in 2-5 ml Enzyme Buffer (EB)* to remove the fixative and digested in 1-2ml Enzyme Solution (ES)* at 37ºC until the material is soft, usually 90 – 120 minutes;
- One root-tip or one coleoptil were dissected per slide into 65% acetic acid to disperse the cells; coverslip was apply to the material without trapping air bubbles and the material was carefully disperse by covering with filter paper and by tapping the coveslip with a needle;
- The slide were checked under a phase contrast microscope and those selected were exposed to compressed CO_2 and the coverslip was then flick off with a razor blade.

The fluorescent in situ hybridization (FISH) technique was adapted from Schwarzacher & Heslop-Harrison (2000) as following described:

- Selected squashes were registered and treated with 200 µl of a solution 1:75 pepsin/0,01 M HCl covered with plastic coverslip for 10 min at 37ºC in a humid chamber;
- After washed 3x 5min with 2xSSC*, the slides were treated with 200 µl of a solution 1:100 RNAase (Ribonuclease A, R-9134, Sigma) /2xSSC covered with a plastic coverslip at 60 min at 37ºC in a humid chamber;
- After washed 3x 5min with 2xSSC, the slides were dehydrated through 70% ethanol per 3 minutes, following by 100% ethanol per 3 minutes and air dry at least 30 minutes.

* Enzyme buffer (10x): 40 ml 100 mM citric acid + 60 ml 100 mM tri-sodium-citrate (10x stock, adjust to pH4.8). Store stock solution at 4 °C. (for work solution dilute 1:10 in water).
Enzyme solution: mixture of 1.8% (w/v) cellulase from Aspergillus niger (Calbiochem, 21947, 4000units/g; final concentration: 80 units/ml), 0.2% 'Onozuka' RS cellulase (5000 units/g, final concentration 10units/ml), and 3% (v/v) pectinase from Aspergillus niger (solution in 40% glycerol, Sigma P4716, 450units/ml; final concentration 13.5units/ml). Make up in 1x enzyme buffer. Store in aliquots at –20 °C.
2x SSC: dilute from 20x SSC stock. (20x SSC consists of 3M sodium chloride and 300mM Trisodium citrate)
RNase 10 mg/ml solution of DNase-free ribonuclease A (Sigma R4642, 70 units/mg) in 10 mM Tris-HCl, pH 8. Store at –20 °C in aliquots.

- To obtain the hybridization solution, the reagents were mixed as follows:

Reagent		Final concentration/amount in hybridization mix	Amount for 1 slide
100% Formamide		50%	20 µl
20x SSC		2x	2 µl
50% Dextran sulphate		10%	8 µl
Salmon sperm DNA 1µg/µl		1µg	1 µl
Probes*	Bio		2-6µl
10% SDS		0.125%	0.5 µl
Sterile water			up to final volume
Total			40µl

* The following probes labeled with biotine (Bio) were used: pTa71, a 9 kb EcoRI fragment of the rDNA unit from wheat (*Triticum aestivum*), containing the 5.8S, 18S, 25S and non-transcribed spacer sequences (Gerlach and Bedbrook, 1979), provided by Genetic Department (2 µl per slide); and 45S rDNA probe that resulted from the amplification of three rDNA different fragments from the pTa71 plasmid: two fragments located on 25S (with 1043 and 1186bp) and one fragment located on 18S (with 800bp) (Fig. 2). Those rDNA fragments were obtained from independent amplification reactions, using the following primers: 25S1 for CTTAGTAACGGCGAGCGAAC and 25S1rev CACTTGGAGCTCTCGATTCC, 25S2 for AACTCACCTGCCGAATCAAC and 25S2rev GCCGAAGCTCCCACTTATC, and 18S for ACTGTGAAACTGCGAATGG and 18Srev, CCCGACTGTCCCTGTTAATC (3 µl per slide,of a 1:1:1 mixture of each fragment).

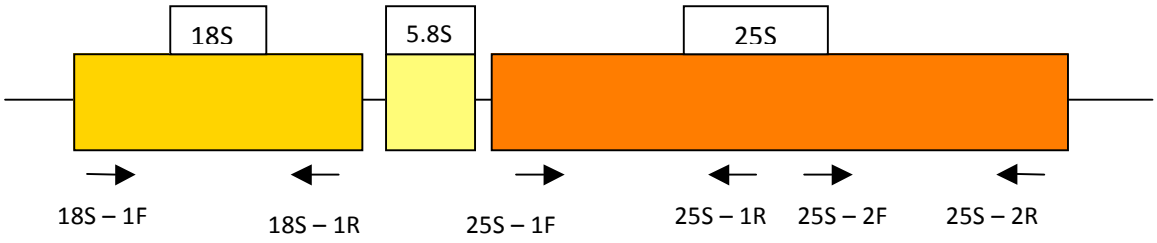

Figure 2. – Schematic representation of the 45S rDNA probe: fragments 18S, 25S1 and 25S2

- The hybridization mixture was denatured at 100ºC for 10 minutes in a boiling water bath, incubated in ice 5 minutes and distributed for the slides (40 µl each), cover with coverslip;
- After that, all slides were place in a Thermocycler programmed to increase the temperature until 77.5ºC, maintain for 5 min, and then decrease the temperature slowly until 37ºC. The slides were maintained in a humid chamber at 37ºC overnight;
- Pós-hybridation washes were done as follows: 2xSSC for 3 minutes at 42ºC; formamide 20% in 0.1xSSC at 42ºC for 10 minutes; 2xSSC 2x 5 minutes at 42ºC; 2xSSC 2x 5 minutes at room temperature (RT); and 4xSSC/tween (0.2%)* 2x 5 minutes at RT;
- Prior to detection, the slides were blocked with a solution of BSA* 5% in 4xSSC/Tween, 200µl/slide cover with a coverslip 5 minutes at RT;
- Detection was performed with 50 µl of a solution of Streptavidin Cy3 conjugated (1/200) in BSA 5% in 4xSSC/Tween with coverslip at 37ºC in humid chamber for 1 hour;
- Following the incubation period, the slides were washed in 4xSSC/Tween 3x 5 minutes.
- To conclude DNA was stained with DAPI* (15µl/slide) covered with glass coverslip and stored in dark overnight.
- The squashes were visualized in an epifluorescence microscopy (Leitz Biomed) using the appropriate filters to observe the DNA stained by DAPI in blue and rDNA signals in red.

The evaluation of the rDNA organization was performed in at least 3 slides of each tissues / treatment and at least 50 nucleus per slide were considered. The comparison of the nuclei frequencies presenting different organization patterns was performed using Chi Test and the average number of rDNA signals per nuclei was compared using Student's t Test.

*4x SSC/ Tween (Detection buffer): 4x SSC (dilute from 20x SSC) containing 0.2% (v/v) Tween 20
BSA - Bovine serum albumin (Sigma B-8894)
DAPI (4',6-diamidino-2-phenylindole, Sigma). Prepare DAPI stock solution of 100 µg/ml in water

Results

The use of Fluorescent In Situ Hybridization allowed the characterization of 45S rDNA topology patterns in root-tips and coleoptiles interphase nuclei from rye plants exposed to high temperature treatment (HTS) and control.

The FISH experiments were performed using two distinct rDNA probes, as described in Material and Method:

- pTa71: 9 kb EcoRI fragment of the rDNA unit from wheat (*Triticum aestivum*), containing the 5.8S, 18S, 25S and non-transcribed spacer sequences;
- a probe designated 45S rDNA probe, that correspond to the amplification of three rDNA different fragments from the pTa71 plasmid: two fragments located on 25S and one fragment located on 18S, that were used in a mixture proportion of 1:1:1.

Both rDNA probes were tested in ice treated c-metaphase root-tip cells and no differences were observed between the in situ hybridization signals obtained. Therefore, both probes were used to evaluate rDNA organization patterns in interphase nuclei.

Concerning rDNA organization pattern, interphase nuclei were classified as:

- Condensed (cond) – nuclei with two condensed rDNA knobs;
- Decondensed (decond) – nuclei with more than two condensed knobs, with decondensed rDNA in situ signals, and with additional linear decondensed in situ signals.

In addition, the average number of rDNA in situ signals per nucleus was also determined.

Root-tip interphase nuclei

The 170 meristematic interphase nuclei from control rye plants (Table 1) corresponded to 66.5% presenting a condensed organization pattern and 33.5% presenting a decondensed one. Condensed nucleus showed two rDNA knobs presenting a similar round form and dimension (Fig. 3 a and b). The nuclei classified as decondensed correspond most often to nuclei presenting irregular and less compact knob and also to nuclei with more than two rDNA blocks. The average rDNA block number was 2.12, due to some decondensed nucleus that presented more than 2 blocks per nuclei.

Table 1. Root-tip interphase cells

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	Control		Control Ice treated		HTS	
Interphase nuclei presenting different rDNA organization pattern						
	cond	decond	cond	decond	cond	decond
Number	113	57	71	40	36	131
Frequence	66.5%	33.5%	64.0%	36.0%	21.6%	78.4%
Total number	170		139		167	
Chi test p			0.646496421		1.03139E-16	
Average number of rDNA blocks / nuclei						
Average	2.12		2.14		2.38	
Standard deviation	0.43		0.38		0.71	
T-student test p			0.698845687		3.01456E-05	

Note: control ice treated results were obtained from only two different root-tips.

The 167 interphase nuclei analyzed from plants subjected to high temperature stress (Table 1), revealed only 21.6% condensed nuclei and 78.4% presenting decondensed patterns. The decondensed phenotype comprehend several organization states: (i) nuclei with rDNA blocks presenting irregular texture and form (Fig. 3 c and d); (ii) nuclei where decondensed in situ signals are observed as highlighted tail emanating from the blocks (Fig. 3e,f); and (iii) nuclei with more than two in situ block signals, corresponding this phenotype to almost half of the nuclei analyzed (Fig. 3 g and h). The average number of rDNA in situ signals per nucleus was 2.38, being the maximum number of blocks observed per nuclei of five.

The statistical analysis, using Chi Test, of the frequencies presented for nuclei from control and HTS plants revealed their significant difference (p=1.03139E-16). Moreover, the statistical analysis, using Student's t-Test, of the average number of rDNA in situ signals per nucleus revealed also significant differences (p=3.01456E-05) between control and HTS root-tip interphase nuclei.

Interphase nuclei from ice treatment root-tip were also analyzed (Table 1). Both the average number of blocks per cell and the distribution of condensed and decondensed nuclei were similar to the ones obtained for control nuclei without ice treatment, since no significant differences were detected through statistical analysis of the results presented. Out of 139 nuclei analyzed, 64.0% presented a

condensed organization pattern and 36.0% presented a decondensed one, and the average rDNA blocks number was 2.14.

Coleoptile interphase nuclei

From control rye coleoptiles, 180 nuclei were analyzed (Table 2), presenting 66.5% condensed pattern and 33.5% a decondensed one. Condensed rDNA knobs were similar to the ones observed in root-tip interphase nuclei, but revealed a less decondensed state (Fig. 3 i and j). The decondensation fraction corresponds to nuclei presenting more decondensed blocks and also to nuclei with more than two blocks, although with smaller expression comparing to what was observed in control root-tip interphase nuclei. The average rDNA blocks number calculated for control coleoptile nuclei was 2.05.

Table 2. Coleoptile interphase cells

	Control		HTS	
Interphase nuclei presenting different rDNA organization				
	cond	decond	cond	decond
Number	112	68	31	95
Frequence	66.5%	33.5%	24.6%	75.4%
Total number		180		126
Chi test p				8.52972E-11
Average number of rDNA blocks / nuclei				
Average	2.05		2.06	
Standard deviation	0.22		0.24	
T-student test p			0.620594577	

Concerning rye HTS coleoptile (Table 2), 126 nuclei were analyzed, revealing 24.6% condensed and 75.4% decondensed nuclei. It must be noted that in HTS coleoptile decondensed nuclei a considerable fraction of rDNA blocks presented higher dimension and a granular texture never observed in root-tip interphase nuclei, being less frequent nuclei presenting more than two rDNA blocks (Fig. 3 l and m). This lower frequency is reflected in the average blocks number calculated for HTS coleoptile nuclei was 2.06, similar to the one obtained for control coleoptile interphase nuclei.

As observed in root-tip nuclei, the statistical analysis of the frequencies presented for coleoptile nuclei from control and HTS rye plants, using Chi Test, revealed a significant difference (p=8.52972E-11).

However, the statistical analysis, using Student's t-Test, of the average number of rDNA in situ signals per nucleus revealed no significant differences (p=0.620594577) between coleoptile interphase cells from control and HTS plants.

The comparison between root-tip and coleoptile interphase cells for control and HTS revealed no significant differences in the parameters considered (Table 3).

Table 3. Root-tip vs. Coleoptile interphase cells

	Control	HTS
Interphase nuclei presenting different rDNA organization		
	Control	HTS
Chi test p	0.403377298	0.521616251
Average number of rDNA blocks / nuclei*		
	Control	HTS
T-student test p	0.024361253	9.3125E-08

Figures

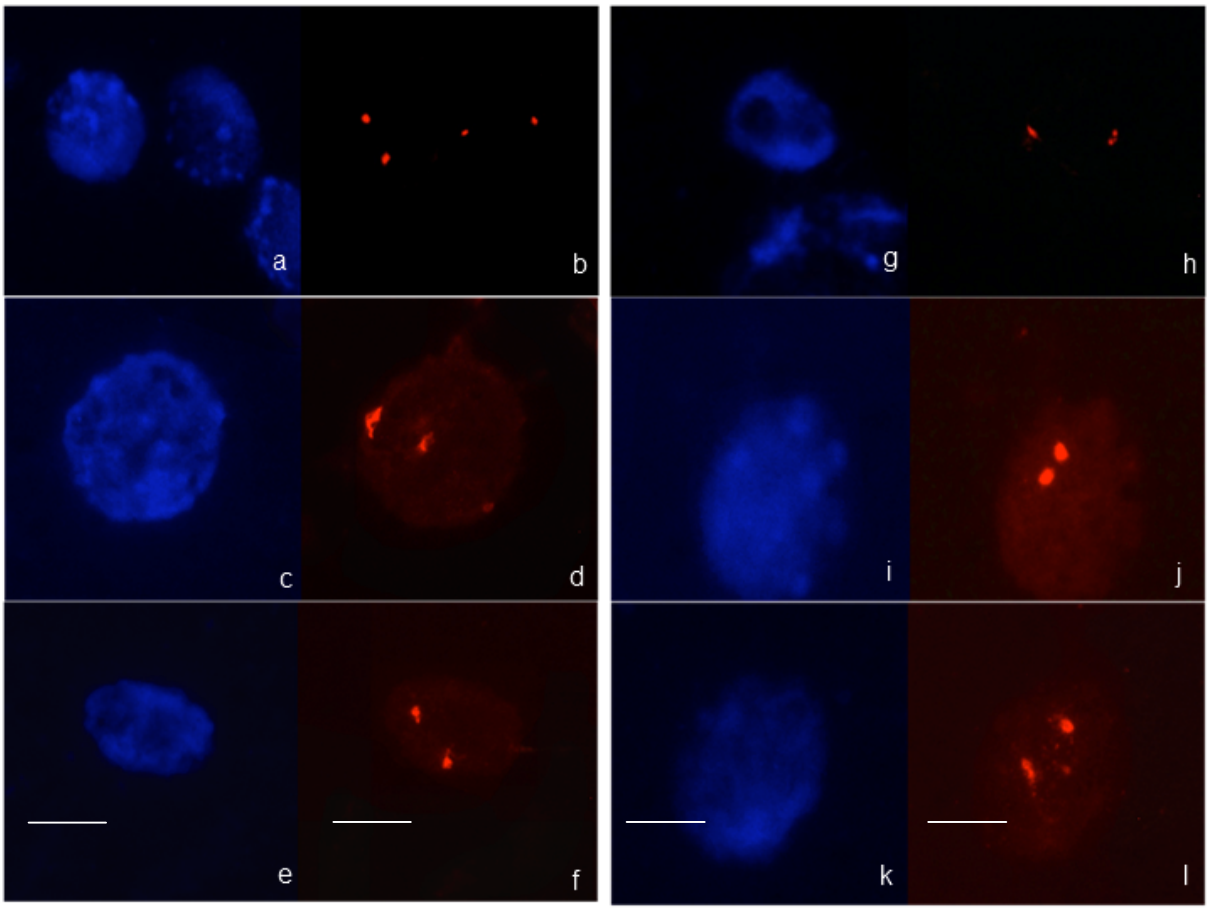

Figure 3.
a,b - Root-tip control interphase nuclei; DAPI (blue) and rRNA probe (red) Bar, 5 µm.
c, d, e, f, g, h - Root-tip HTS interphase nuclei; DAPI (blue) and rRNA probe (red) Bar, 5 µm.
i, j - Coleoptilo control interphase nuclei; DAPI (blue) and rRNA probe (red) Bar, 5 µm.
k, l - . Coleoptilo control interphase nuclei; DAPI (blue) and rRNA probe (red) Bar, 5 µm.

Discussion and conclusions

The main aim of this work was to characterize the interphasic organization patterns of ribosomal chromatin in rye exposed to high temperature stress in comparison to control. This analysis was performed using in situ hybridization with rDNA probes in different tissues: meristematic root-tip cells and coleoptile cells from the coleoptile.

Beyond pTa71, that corresponds to a wheat entire rDNA unit and is the traditionally used as rDNA probe, in this study a new probe to detect rDNA was created by drawing primers to amplify and simultaneously label three rDNA fragments: two fragments located on 25S and one fragment located on 18S. This probe was confirmed to hybridize exclusively to the rDNA *loci* in rye 1R nucleolar chromosomes in root-tip c-metaphasic cells and yielded equal results in both tissues analyzed from plantlets maintained in the different growth conditions analyzed. Thus, this rDNA 45S probe, used for the first time, can replace pTa71 in in situ hybridization experiments successfully and with the advantage of being labeled and amplified by PCR at the same time being therefore easier to prepare.

To evaluate the interphasic organization patterns of ribosomal chromatin, as showed in Results, two parameters were considered: the frequency of nuclei with condensed and decondensed organization patterns and the average number of rDNA in situ signals per nuclei.

The analysis of those two parameters clearly demonstrated that the high temperature treatment induces rDNA chromatin decondensation, since the statistical analysis of the results revealed in both root-tip and coleoptile nuclei significant differences, which correspond to:

- an increase in the frequencies of nuclei showing decondensed rDNA organization patterns;
- an increase in the average number of rDNA in situ signals per nuclei.

The first parameter represents the classification of interphase nuclei in different chromatin organization types, being considered as decondensed nuclei with: rDNA blocks presenting irregular texture and form; decondensed in situ signals as highlighted tails emanating from the rDNA blocks; and with more than two in situ block signals. In situ hybridization signals exceeding two per nuclei, that correspond to the perinucleolar knobs of the two NOR *loci* (Morais-Cecílio *et al.*, 2000), may correspond to more decondensed rDNA regions, as was demonstrated to occur in wheat (Silva *et al* 2008), where condensed ribosomal chromatin regions inside the nucleolus were showed to colocalize with BrUTP incorporation, demonstrating them to be transcriptionaly active.

The increase in the average number of in situ rDNA signals per nuclei can be interpreted also as revealing an increase decondensation of ribosomal chromatin. In fact, the observed increase in the number of in situ hybridization signals reflect intercalary euchromatic decondesations flanked by condensed heterochromatin rDNA blocks as was observed in the cytological evaluation of rDNA chromatin organization in *Arabidopsis* (Early *et al*., 2006).

Although in both root-tip and coleoptile nuclei the high temperature treatment induced rDNA decondensation revealed by an increase in the frequency of decondensed nuclei, the correspondent organization patterns were qualitatively different. The HTS coleoptile nucleus showed a pattern of larger rDNA blocks showing a granulose texture that reveals a different chromatin organization pattern, contrary to decondensed root-tip nucleus, where this pattern was never observed. This variation seems to be related with the plantlet tissue considered being coleoptile interphasic nuclei response to HTS in terms of the rDNA chromatin organization different in comparison to root-tip nuclei. As described in Pontes *et al*. (2007) during *Arabidopsis* development, the rDNA organization patterns revealed by in situ hybridization undergo changes, correlated with different epigenetic patterns and with different expressions levels.

As described in results and above, the class of decondensed nuclei correspond to different patterns of rDNA organization. Thus, in all cases analyzed, more than one kind of decondensation phenotype is observed in the same tissue. This situation can be due to different exposures of the cells to the high temperature treatments, either in terms of cell situation in the tridimensional organ structure or in terms of the cell cycle phase considered. Those different cells may have different behaviors in HTS response concerning rDNA chromatin organization.

Epigenetic pathways are strongly linked to the establishment and maintenance of certain states of ribosomal chromatin organization. Recent findings like artificial induced DNA hypomethylation, proved to be correlated with modifications of rye rDNA chromatin organization and transcription levels (Caperta *et al.*, 2007). Additionally, Somers *et al*. (1992) demonstrated the increased of the transcription of several genes, namely genes coding for 150 heat shock proteins, induced by 40ºC treatments for 2 hours, revealed by the heat shock protein profiles of wheat and rye. Thus, epigenetic pathways have a decisive role in plant response to stress and our results obtained in rye emphasize the role of chromatin organization modulation in plant stress response. Since the parameters evaluated in this study – frequencies of nuclei with different rDNA organization patterns and average number of rDNA in situ signals per nuclei – did not reveal the qualitative differences described between the decondensed organization patterns of root-tip and coleoptile interphase nuclei, since the comparison of those parameters did not reveal significant**,** in the future image quantification of those interphase nuclei will be performed using the software ImageJ. This analysis will allow the quantification of the rDNA in situ signals obtained in comparison with the nuclear dimension for each tissue / growth conditions and will unravel the quantification of more subtle differences between the organization patterns detected.

Our findings open some new perspectives. In this context, the next step will be the quantification of rDNA transcripts by quantitative real-time PCR, to search for correlations between rDNA decondensation induced by high temperature stress and ribosomal genes transcription levels. In addition, the cytological evaluation of chromatin organization as well as the molecular evaluation of transcription levels will be extended to other sequences, namely rye non-coding repetitive sub-telomeric sequences (pSc200).

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